Differential Expression of Ionic Channels in Rat Anterior Pituitary Cells

Fredrick Van Goor, Dragoslava Zivadinovic, and Stanko S. Stojilkovic

Endocrinology and Reproduction Research Branch
National Institute of Child Health and Human Development
National Institutes of Health
Bethesda, Maryland 20892-4510

Secretory anterior pituitary cells are of the same origin, but exhibit cell type-specific patterns of spontaneous intracellular Ca^{2+} signaling and basal hormone secretion. To understand the underlying ionic mechanisms mediating these differences, we compared the ionic channels expressed in somatotrophs, lactotrophs, and gonadotrophs from randomly cycling female rats under identical cell culture and recording conditions. Our results indicate that a similar group of ionic channels are expressed in each cell type, including transient and sustained voltage-gated Ca^{2+} channels, tetrodotoxin-sensitive Na^+ channels, transient and delayed rectifying K^+ channels, and multiple Ca^{2+}-sensitive K^+ channel subtypes. However, there were marked differences in the expression levels of some of the ionic channels. Specifically, lactotrophs and somatotrophs exhibited low expression levels of tetrodotoxin-sensitive Na^+ channels and high expression levels of the large-conductance, Ca^{2+}-activated K^+ channel compared with those observed in gonadotrophs. In addition, functional expression of the transient K^+ channel was much higher in lactotrophs and gonadotrophs than in somatotrophs. Finally, the expression of the transient voltage-gated Ca^{2+} channels was higher in somatotrophs than in lactotrophs and gonadotrophs. These results indicate that there are cell type-specific patterns of ionic channel expression, which may be of physiological significance for the control of Ca^{2+} homeostasis and secretion in unstimulated and receptor-stimulated anterior pituitary cells. (Molecular Endocrinology 15: 1222–1236, 2001)

0888-8809/01/$3.00/0
Molecular Endocrinology 15(7): 1222–1236

INTRODUCTION

The anterior pituitary is composed of the five major hormone-secreting cell types, corticotrophs, lactotrophs, thyrotrophs, somatotrophs, and gonadotrophs. Corticotrophs arise from a lineage that is distinct from the other cell types, whereas the remaining cell types share common transcription factors and frequently produce multiple hormones, indicating that they are closely related (1). Despite their similar origin, somatotrophs, lactotrophs, and gonadotrophs differ with respect to their pattern of spontaneous electrical activity, intracellular Ca^{2+} ($[Ca^{2+}]_i$) signaling, basal hormone secretion, and neuroendocrine regulation of spontaneous Ca^{2+} influx and Ca^{2+}-dependent hormone secretion. Specifically, somatotrophs and lactotrophs exhibit extracellular Ca^{2+}-dependent, high-amplitude $[Ca^{2+}]_i$ transients, whereas only low-amplitude $[Ca^{2+}]_i$ signals have been observed in unstimulated gonadotrophs (2, 3). In parallel to spontaneous $[Ca^{2+}]_i$ signaling, somatotrophs and lactotrophs exhibit high basal secretion, whereas basal gonadotropin secretion is low and not dependent on extracellular Ca^{2+} (4). Consistent with this, Ca^{2+} signaling and secretion in somatotrophs and lactotrophs, but not in gonadotrophs, are under dual control by positive and negative hypothalamic factors (5–7). These differences suggest that, despite the similar origin of somatotrophs, lactotrophs, and gonadotrophs, there may be differences in the ionic channels expressed in the three hormone-secreting cell types.

Many of the ionic channels in native and immortalized anterior pituitary cells have been characterized previously, including voltage-gated Ca^{2+} channels (VGCCs), tetrodotoxin (TTX)-sensitive and insensitive Na^+ channels, voltage-gated K^+ channels, Ca^{2+}-controlled K^+ channels, Cl^- channels, and nonselective cationic channels (2, 3). However, it is difficult to directly compare the expression levels and the properties of the individual ionic channels from the different studies, due to differences in the species, sex, and hormonal status of the animals used, as well as the cell

cultures and recording conditions. Because of this, it is still not known whether differences in the expression levels and/or ionic channel properties underlie the cell type-specific patterns of voltage-gated Ca^{2+} entry and hormone secretion. To address this problem, we compared the expression levels and voltage-dependent properties of the ionic channels in somatotrophs, lactotrophs, and gonadotrophs from randomly cyclic female rats under identical culture and recording conditions. A similar group of ionic channels is observed in all three cell types. However, there was a marked difference in the functional expression of the individual ionic channels between the three hormone-secreting cell types. These differences likely underlie the cell type-specific patterns of $[Ca^{2+}]_i$ signaling and hormone secretion observed in unstimulated somatotrophs, lactotrophs, and gonadotrophs.

RESULTS

Voltage-Gated Na^+ Channels

The functional expression and voltage-dependent properties of the Na^+ channels in rat somatotrophs, lactotrophs, and gonadotrophs were examined under isolated Na^+ current (I_{Na}) recording conditions using the conventional whole-cell technique. In rat somatotrophs and lactotrophs, a rapidly activating and inactivating I_{Na} was observed at membrane potentials more depolarized than −37 mV and reached maximum amplitude around −7 mV (Fig. 1, A, B, and D). A similar I_{Na} was observed in rat gonadotrophs (Fig. 1C), but its peak amplitude was much greater and its current-voltage relation was shifted approximately 10 mV in the hyperpolarizing direction compared with that for

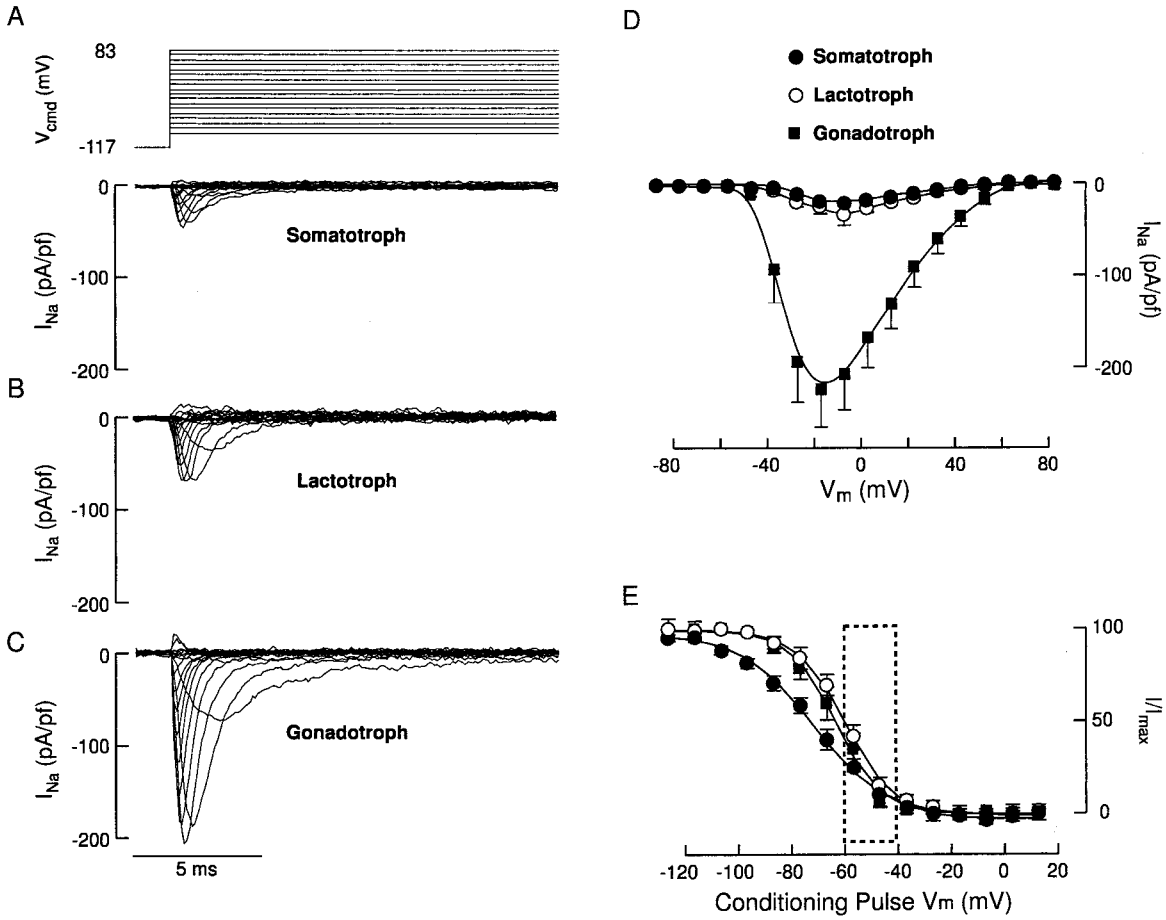

Fig. 1. Voltage-Gated Na^+ Channels in Somatotrophs, Lactotrophs, and Gonadotrophs

Representative voltage-gated I_{Na} traces in somatotrophs (panel A, n = 5), lactotrophs (panel B, n = 5), and gonadotrophs (panel C, n = 5) elicited by 100-msec voltage steps from −87 mV to 83 mV form a holding potential of −117 mV. D, Current-voltage relation of the voltage-gated I_{Na} in all three cell types. E, Steady-state inactivation curves for the voltage-gated I_{Na} in all three cell types were generated by stepping the membrane potential to between −127 and −13 mV for 200 msec before stepping to a 100-msec command potential of −17 mV (holding potential = −97 mV). The peak I_{Na} evoked during the command potential to −17 mV in each cell type were normalized to the maximum inward current and plotted against the conditioning pulse potentials. In this and the following figures, the ionic currents were normalized to the membrane capacitance of each cell examined to compensate for the differences in the size of cells. The *dashed box* in this figure and in Figs. 3 and 5 represents the range of baseline potentials commonly observed in spontaneously active somatotrophs, lactotrophs, and gonadotrophs (54).

somatotrophs and lactotrophs (Fig. 1D). In somatotrophs, lactotrophs, and gonadotrophs, application of 1 μM TTX reduced the peak I_{Na} amplitude by 99.4 ± 0.7% (n = 5), 98.2 ± 3.3% (n = 5), and 98.9 ± 1.2% (n = 5), respectively.

To determine the proportions of the TTX-sensitive I_{Na} in each cell type that are available for activation at different resting membrane potentials, the steady-state inactivation properties of the I_{Na} in all three cell types were examined using a two-pulse protocol. This protocol consisted of a series of 200-msec conditioning pulses from −127 mV to −13 mV, followed by a 100-msec test pulse to −17 mV (holding potential = −97 mV). The peak I_{Na} evoked during the test pulse was normalized to the maximal inward current and plotted against the conditioning pulse potentials, and the resulting curves were fitted with a single Boltzmann relation (Fig. 1E). In somatotrophs, the membrane potential at which there is 50% of the maximal current ($E_{1/2}$) available for activation is −72 mV. In lactotrophs and gonadotrophs, the $E_{1/2}$ values were similar and were approximately 10 mV more depolarized than that observed in somatotrophs.

Voltage-Gated Ca^{2+} Channels

The functional expression and voltage-dependent properties of the Ca^{2+} channels in rat somatotrophs, lactotrophs, and gonadotrophs were examined under isolated Ca^{2+} current (I_{Ca}) recording conditions using the conventional whole-cell technique. The I_{Ca} in each cell type was examined by applying a series of 400-msec depolarizing voltage steps from −97 mV to +83 mV in 10-mV increments (holding potential = −97 mV). In all three cell types, a transient I_{Ca} was observed in response to the depolarizing voltage steps. In somatotrophs, the transient I_{Ca} was activated by voltage steps more depolarized than −77 mV and reached maximum amplitude between −27 and −17 mV (Fig. 2, A and B). In the other two cell types, the activation threshold of the transient current was shifted approximately 10 mV in the depolarizing direction (Fig. 2, A and B). In addition, the peak of the transient I_{Ca}-voltage relationship was shifted more than 20 mV in the depolarizing direction compared with that in somatotrophs (Fig. 2B). To determine the density of the transient I_{Ca} in each cell type, the peak

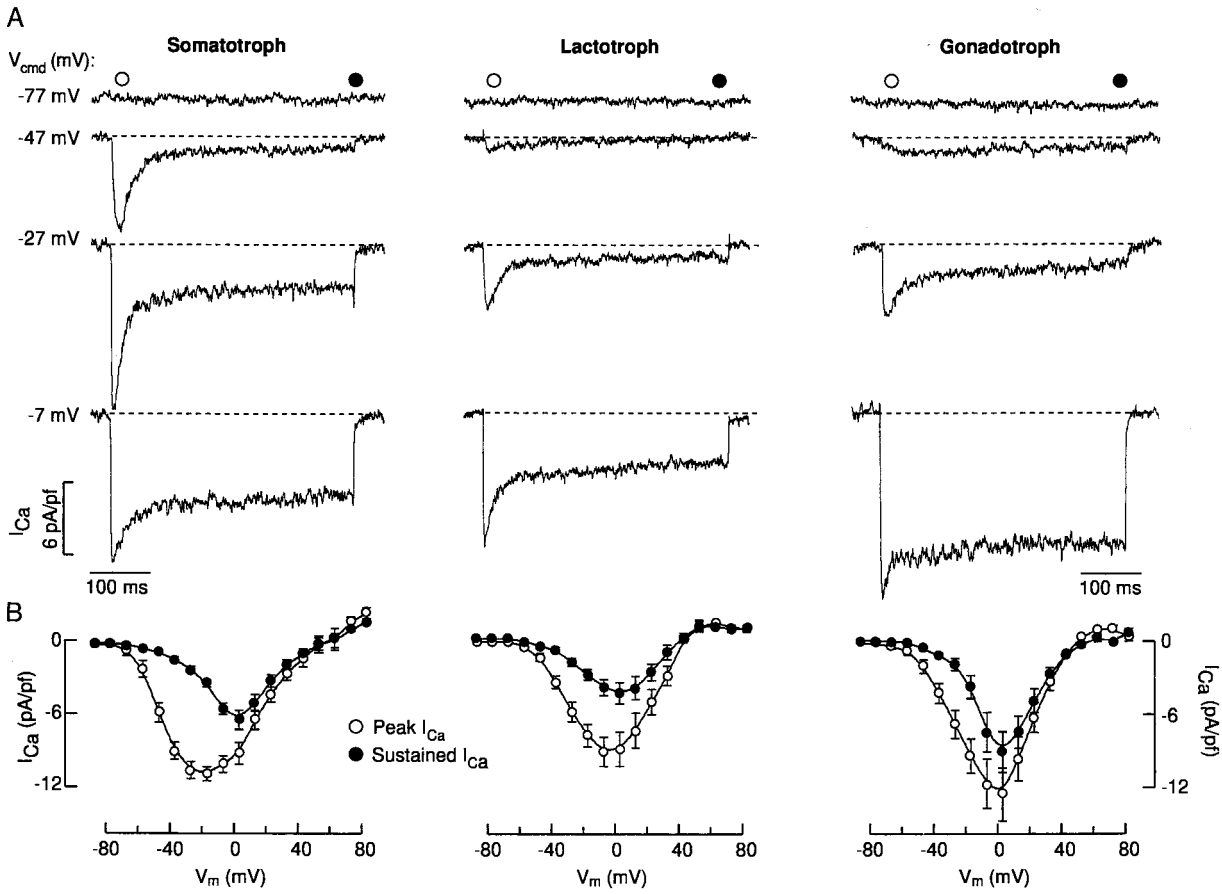

Fig. 2. Voltage-Gated Ca^{2+} Channels in Somatotrophs, Lactotrophs, and Gonadotrophs

A, Representative voltage-gated I_{Ca} traces in somatotrophs (n = 20), lactotrophs (n = 16), and gonadotrophs (n = 14) elicited by 400-msec voltage steps from a holding potential of −97 mV to −77, −47, −27, and −7 mV are shown. B, Current-voltage relation of the peak (*open circles,* 0–25 msec) and sustained (*filled circles,* 390–400 msec) voltage-gated I_{Ca} in all three cell types.

I_{Ca} evoked by voltage steps to −47 mV (holding potential = −97 mV) was analyzed. This membrane potential was used because it activates a transient I_{Ca} in all three cell types with only minimal activation of a sustained I_{Ca} (Fig. 2A). The mean peak transient I_{Ca} densities at −47 mV in somatotrophs, lactotrophs, and gonadotrophs were −6.6 ± 0.8 picoamperes/picofarads (pA/pF) (n = 20), −2.2 ± 0.3 pA/pF (n = 16), and −2.7 ± 0.6 pA/pF (n = 14), respectively.

The transient I_{Ca} in somatotrophs, lactotrophs, and gonadotrophs was followed by slow-inactivating I_{Ca}. In all three cell types, the sustained I_{Ca} was activated by voltage steps more depolarized than −57 mV and reached a maximum amplitude around +3 mV (Fig. 2, A and B). In addition to the similar current-voltage relationship of the sustained I_{Ca}, dihydropyridine agonists and antagonists had a similar effect on the sustained I_{Ca} amplitude in all three cell types (Table 1). To compare the density of the slow-inactivating I_{Ca} in each cell type, the sustained I_{Ca} (390–400 msec) evoked by voltage-steps to +3 mV was analyzed. In somatotrophs, lactotrophs, and gonadotrophs the mean sustained I_{Ca} densities were −6.8 ± 0.8 pA/pF (n = 20), −4.5 ± 0.9 pA/pF (n = 16), and −9.2 ± 1.7 pA/pF (n = 14), respectively.

To determine the proportions of the total voltage-gated I_{Ca} in each cell type that is available for activation at various different resting membrane potentials, the steady-state inactivation properties of the I_{Ca} were examined using a two-pulse protocol. This protocol consisted of a conditioning pulse ranging from −127 mV to −13 mV for 400 msec, after which a 200-msec test pulse to −7 mV was applied (Fig. 3, A–C). The normalized test current in each cell type was plotted against the conditioning pulse potentials, and the resulting curve was fitted with a single Boltzmann relation (Fig. 3D). In somatotrophs, $E_{1/2}$ was −56 mV (slope factor = 6.5). The $E_{1/2}$ in lactotrophs and gonadotrophs were −34 mV (slope factor = 7) and −40 mV (slope factor = 4), respectively, which were more than 15 mV more depolarized than that in somatotrophs. The more pronounced steady-state inactivation of the total I_{Ca} in somatotrophs compared with that in lactotrophs and gonadotrophs is consistent with the greater expression of the transient I_{Ca} in somatotrophs.

Voltage-Gated K^+ Channels

The functional expression and voltage-dependent properties of the voltage-gated K^+ channels in rat somatotrophs, lactotrophs, and gonadotrophs were examined under isolated K^+ current (I_K) recording conditions using the perforated-patch technique. To exclude Ca^{2+}-sensitive I_K ($I_{K(Ca)}$), extracellular Ca^{2+} entry through VGCCs was blocked by addition of 200 μM Cd^{2+} to the bath solution. The total voltage-gated I_K in each cell type was examined by the application of a 500-msec holding potential to −130 mV before giving a series of 1.5-sec depolarizing voltage steps from −90 mV to +90 mV (Fig. 4A; *upper panels*). In rat somatotrophs, both the peak (0–25 msec) and sustained (1.4–1.5 sec) I_K were activated at membrane potentials more depolarized than −30 mV. Once activated, the total voltage-gated I_K inactivated slowly during the 1.5-sec depolarizing voltage steps (Fig. 4, A and D). In lactotrophs and gonadotrophs, the peak I_K activated at membrane potentials more depolarized than −50 mV, whereas the sustained I_K activated at membrane potentials more depolarized than −30 mV. Unlike somatotrophs, the total voltage-gated I_K in lactotrophs and gonadotrophs was characterized by a fast- and slow-inactivating I_K (Fig. 4, A and D).

To isolate the slow-inactivating I_K, the transient I_K was eliminated by the application of a 500-msec holding potential to −40 mV before the 1.5-sec depolarizing voltage steps from −90 mV to +90 mV (Fig. 4B). Under these conditions, a slow-inactivating I_K was observed in somatotrophs and lactotrophs (Fig. 4B). A similar slow-inactivating I_K was observed in gonadotrophs in response to voltage steps between −40 and 20 mV. However, a small fast-inactivating I_K was maintained at more depolarized membrane potentials, which may be due to incomplete inactivation of the transient I_K in gonadotrophs by the conditioning pulse. To compare the densities of the slow-inactivating I_K in each cell type, the sustained I_K evoked by voltage steps to +90 mV (holding potential of −90 mV) was analyzed. The sustained I_K density in lactotrophs was 147 ± 15 pA/pF (n = 5), which was significantly ($P < 0.05$) smaller than that in somatotrophs (254 ± 32 pA/pF; n = 5) and gonadotrophs (282 ± 32 pA/pF; n = 8).

Table 1. Effects of L-Type Ca^{2+} Channel Agonists and Antagonists on Sustained I_{Ca} in Somatotrophs, Lactotrophs, and Gonadotrophs

	Somatotrophs (pA/pF)	Lactotrophs (pA/pF)	Gonadotrophs (pA/pF)
Control (5)[a]	9.8 ± 2.0	4.6 ± 2.5	7.9 ± 2.5
1 μM Nifedipine (8)	6.7 ± 1.7[b]	2.5 ± 0.8[b]	3.0 ± 0.5[b]
1 μM Bay K 8644 (8)	17.0 ± 3.7[b]	12.5 ± 0.8[b]	9.2 ± 1.3[b]

All I_{Ca} were evoked by a 200-msec voltage step to −7 mV from a holding potential of −87 mV and are expressed as mean ± SEM. The mean I_{Ca} between 190 and 200 msec are shown.

[a] Number of trials in each treatment group.

[b] Significant difference ($P < 0.05$) compared to controls.

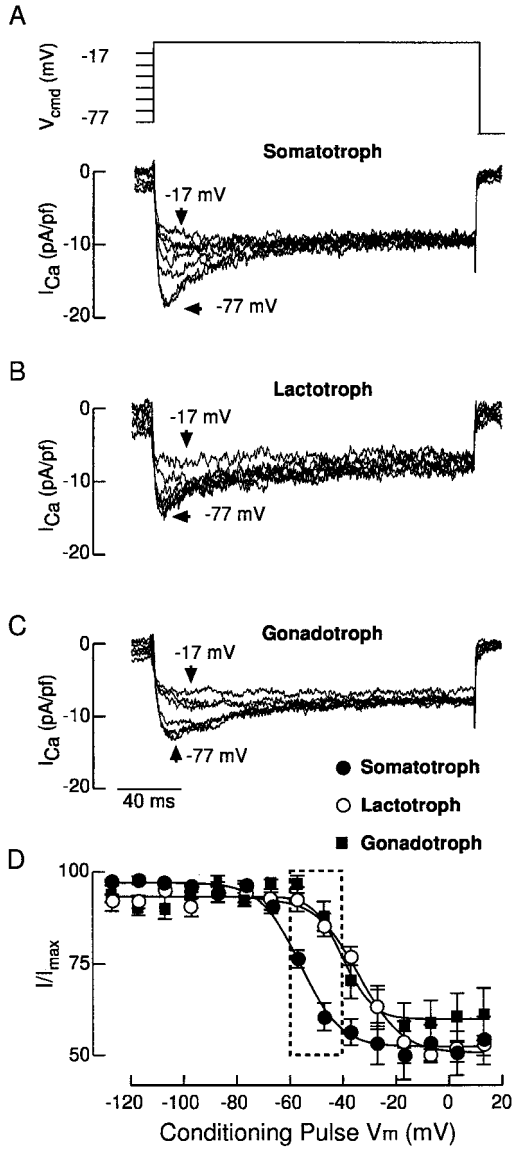

Fig. 3. Steady-State Inactivation of the total I_{Ca} in Somatotrophs, Lactotrophs, and Gonadotrophs

A–C, Steady-state inactivation curves for the voltage-gated I_{Ca} in each cell type were generated by stepping the membrane potential to between −127 and −3 mV for 400 msec before stepping to a 200-msec command potential of −7 mV (holding potential = −97 mV). Representative traces of the remaining current elicited during the command potential after conditioning pulses between −77 mV and −17 mV are shown. D, The peak I_{Ca} evoked during the command potential to −7 mV in all three cell types were normalized to the maximum inward current and plotted against the conditioning pulse potentials. The data for each cell type were fitted with a single Boltzmann relation.

We next compared the transient I_K in each cell type by subtracting the I_K elicited during the various voltage steps after a holding potential to −40 mV from the total I_K (Fig. 4C). In rat somatotrophs, a small transient I_K was evoked by membrane potential steps more depolarized than −30 mV, reaching a peak amplitude of 67 ± 18 pA/pF at +90 mV (n = 5). In contrast, a large transient I_K was observed in lactotrophs (n = 5) and gonadotrophs (n = 8; Fig. 4, C and D; *right panel*). The transient I_K in these cell types activated at membrane potentials more depolarized than −50 mV and reached maximum amplitude at +90 mV of 343 ± 73 pA/pF and 389 ± 82 pA/pF at +90 mV, respectively.

To determine the proportions of the total voltage-gated I_K in each cell type that is available for activation at various different membrane potentials, the steady-state inactivation properties of the I_K were examined using a two-pulse protocol. This protocol consisted of a series of 1.5-sec conditioning pulses from −130 mV to −10 mV, followed by a 400-msec test pulse to +90 mV (Fig. 5A). The representative I_K tracings evoked by the two-pulse protocol are shown in Fig. 5, A–C. The normalized test current in each cell type was plotted against the conditioning pulse potentials (Fig. 5D). A single Boltzmann relation could not be fitted to the data, further indicating the presence of a fast- and slow-inactivating component in each of the three cell types. However, the magnitude of steady-state I_K inactivation in somatotrophs was much less than that observed in the other two cell types, which is consistent with the low expression levels of the transient I_K in somatotrophs.

Ca^{2+}-Activated K^+ Channels

Voltage-gated Ca^{2+} entry was used to examine the expression of $I_{K(Ca)}$ in somatotrophs, lactotrophs, and gonadotrophs. To activate VGCCs, a modified two-step protocol was used. This protocol consisted of an initial 100-msec voltage-step to −10 mV (holding potential = −90 mV) during which VGCCs were activated (Fig. 6A; *left panel*). This Ca^{2+}-influx step was immediately followed by a 500-msec test pulse to +80 mV, during which the evoked I_K was monitored. As +80 mV is near the reversal potential for Ca^{2+} under our experimental conditions, there should be minimal Ca^{2+} entry during this step. Consistent with this, in the absence of the Ca^{2+}-influx step, there was little to no change in $[Ca^{2+}]_i$, whereas in the presence of a Ca^{2+}-influx step alone or in combination with the test pulse there was a marked increase in $[Ca^{2+}]_i$ in all three cell types (Fig. 7). In addition, the I_K evoked by the test pulse to +80 mV in the absence of the Ca^{2+}-influx step was similar in Ca^{2+}-containing and Ca^{2+}-deficient medium (Table 2).

In the presence of a 100-ms Ca^{2+}-influx step, the peak I_K evoked during the test pulse was reduced by extracellular Ca^{2+} removal in somatotrophs, lactotrophs, and gonadotrophs (Fig. 6, A–C, and Table 3), indicating $I_{K(Ca)}$ values are expressed in all three cell types. To compare $I_{K(Ca)}$ activation between the three cell types, the I_K evoked in Ca^{2+}-deficient medium was subtracted from that in Ca^{2+}-containing medium (Fig. 6, A–C, *right panels*). These results clearly indicate that $I_{K(Ca)}$ activation by voltage-gated Ca^{2+} entry was greatest in somatotrophs and smallest in gonadotrophs.

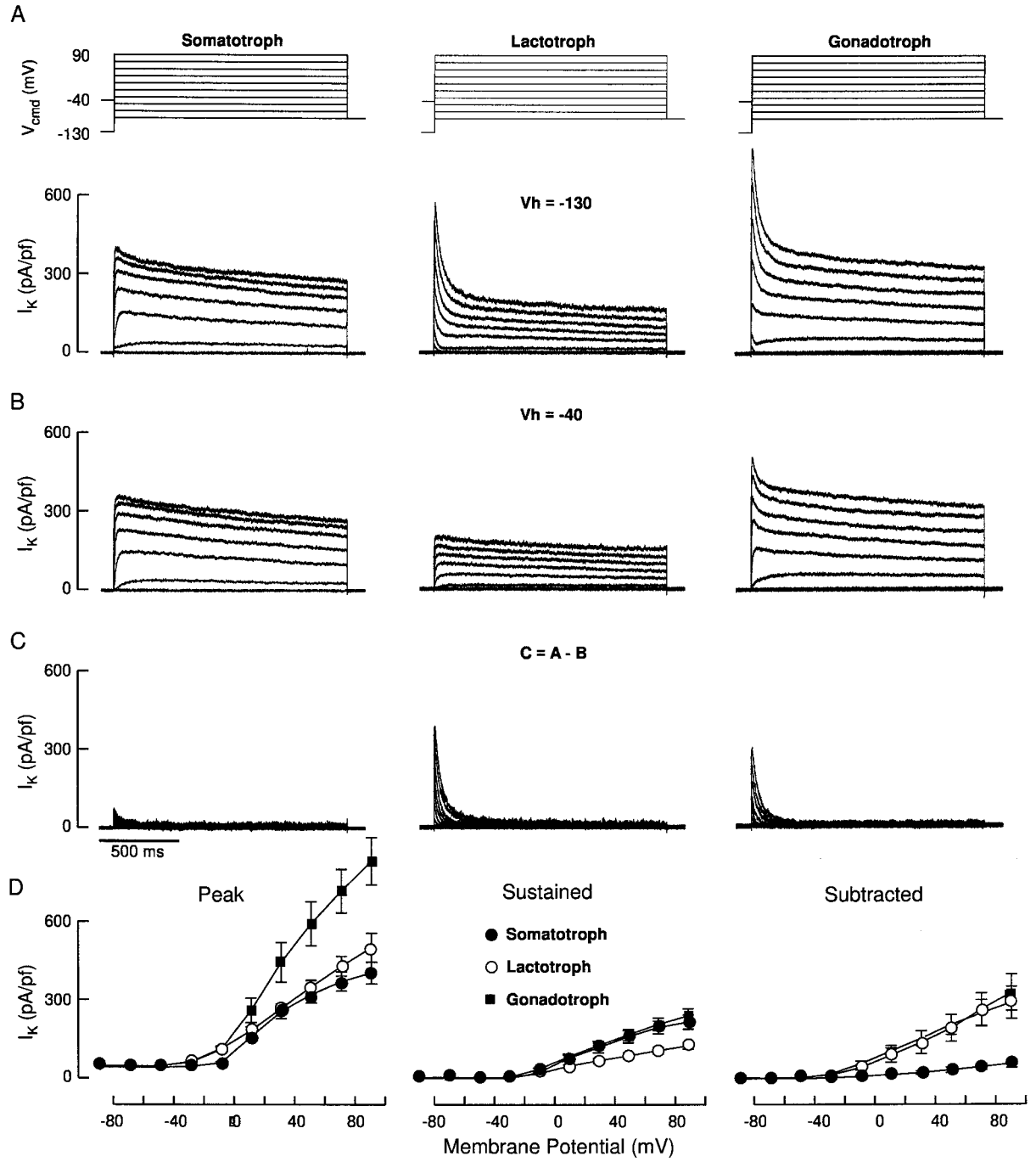

Fig. 4. Voltage-Gated K^+ Channels in Somatotrophs, Lactotrophs, and Gonadotrophs

A, Representative voltage-gated I_K traces in somatotrophs (n = 5), lactotrophs (n = 5), and gonadotrophs (n = 8) elicited by 1.5-sec voltage steps from −90 to 90 mV in 20-mV increments from a holding potential of −130 mV. B, Representative voltage-gated I_K traces in somatotrophs (n = 5), lactotrophs (n = 5), and gonadotrophs (n = 8) elicited by 1.5-sec voltage steps from −90 to 90 mV in 20-mV increments from a holding potential of −40 mV. C, A transient I_K in all three cell types was isolated by a point-by-point subtraction of the current traces in panels A and B. D, Current-voltage relationship of the peak (0–25 msec; *left panel*), sustained (1.49–1.50 sec; *middle panel*) and subtracted (A − B; *right panel*) I_K in all three cell types. To block $I_{K(Ca)}$, 200 μM $CdCl_2$ was added to the bath solution to block voltage-gated Ca^{2+} entry.

The differences in $I_{K(Ca)}$ activation between the three cell types may be due to differences in the ability of the Ca^{2+} influx step to drive voltage-gated Ca^{2+} entry and increase $[Ca^{2+}]_i$. To test this, we compared the increase in $[Ca^{2+}]_i$ evoked by the two-step protocol in each cell type (Fig. 7). In somatotrophs and gonadotrophs, the two-step protocol increased the $[Ca^{2+}]_i$ by 160 ± 18 nM (n = 17) and 164 ± 19 nM (n = 12),

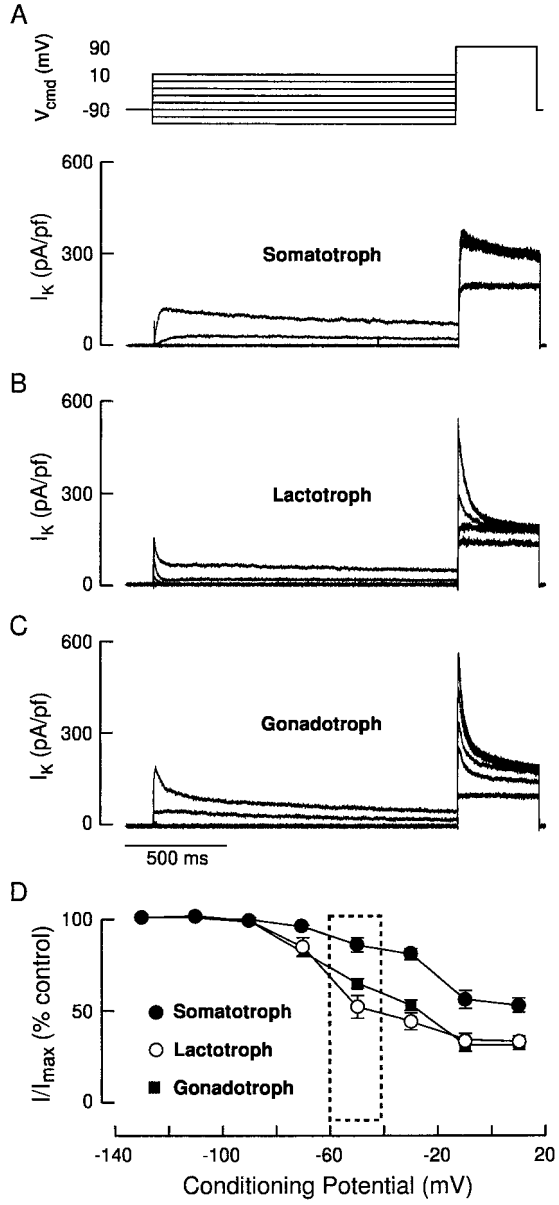

Fig. 5. Steady-State Inactivation of the Total I_K in Somatotrophs, Lactotrophs, and Gonadotrophs

A–C, Steady-state inactivation curves for the peak voltage-gated I_K in each cell type were generated by stepping the membrane potential to between −130 and −10 mV for 1.5 sec before stepping to a command potential of 90 mV (holding potential = −90 mV). Representative traces of the remaining current elicited during the command potential after conditioning pulses to −130, −110, −90, −70, −50, −30, −10, and 10 mV are shown. D, The peak I_K evoked during the command potential to +90 mV all three cell types were normalized to the maximum inward current and plotted against the conditioning pulse potentials.

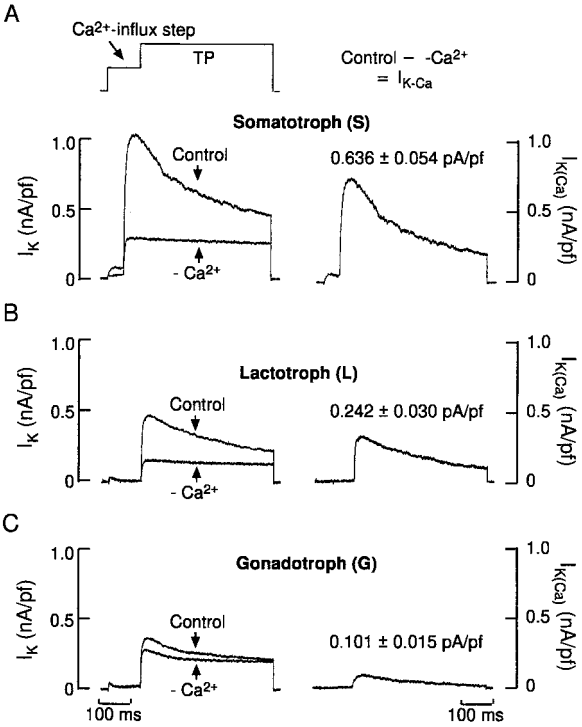

Fig. 6. Identification of $I_{K\text{-}Ca}$ in Somatotrophs, Lactotrophs, and Gonadotrophs

A–C, *Left panels*, Representative I_K traces in somatotrophs (n = 13), lactotrophs (n = 15), and gonadotrophs (n = 9) evoked by the two-step protocol (A, *upper panel*) in Ca^{2+}-containing (*control*) and Ca^{2+}-deficient (−Ca^{2+}) medium. The two-step protocol consisted of a 100-msec condition pulse to −10 mV, to activated voltage-gated Ca^{2+} entry, followed by a 500-msec test pulse to +90 mV, during which the peak I_K was monitored. A–C, *Right panels*, The net $I_{K\text{-}Ca}$ activated by the two-step protocol in each cell type was isolated by subtracting the current evoked in the presence of Ca^{2+}-deficient medium from that in Ca^{2+}-containing medium. The mean ± SEM values of the peak I_{KCa} evoked during the test pulse are shown.

respectively. In lactotrophs, a smaller increase in $[Ca^{2+}]_i$ of 122 ± 13 nM (n = 12) was evoked by the two-step protocol. These results are consistent with the smaller amplitude noninactivating I_{Ca} in lactotrophs compared with the other two cell types (Fig. 2). Nevertheless, these results indicate that the small amplitude $I_{K(Ca)}$ observed in gonadotrophs is not due to the inability of voltage-gated Ca^{2+} entry to increase $[Ca^{2+}]_i$. Therefore, the differences between $I_{K(Ca)}$ activation in the three cell types appears to be due to differences in channel expression and not the capacity of the Ca^{2+}-influx step to drive changes in $[Ca^{2+}]_i$.

The dependence of $I_{K(Ca)}$ activation on voltage-gated Ca^{2+} influx was further confirmed in experiments with dihydropyridine agonist and antagonists. As shown in Fig. 8, A and C, addition of the L type Ca^{2+} channel blocker, nifedipine, significantly decreased the amplitude of the I_K in all three cell types studied. Furthermore, Bay K 8644, an L-type Ca^{2+} channel agonist, significantly increased the amplitude of I_K in all three cell types (Fig. 8, A and C). Consistent with the differential expression of Ca^{2+}-activated K^+ channels in the three cell types, the effects of nifedi-

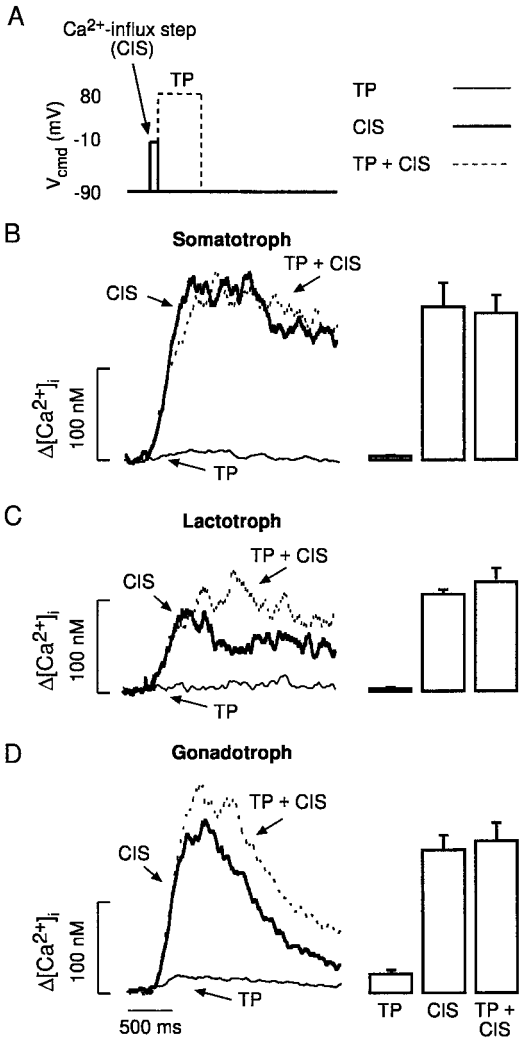

Fig. 7. Profile of $[Ca^{2+}]_i$ in Cells Exposed to One- and Two-Step Protocol.

A–D, Representative $[Ca^{2+}]_i$ traces without (*solid line*) and with (*dotted line*) the application of a 100-msec Ca^{2+}-influx step to −10 mV before the application of a 500-msec step to a test potential (TP) of +80 mV or by the Ca^{2+}-influx step (CIS) alone (*thick line*) from a holding potential = −90 mV in somatotrophs (n = 17), lactotrophs (n = 12), and gonadotrophs (n = 12). The net change in $[Ca^{2+}]_i$ (mean ± SEM) evoked by the TP, CIS, and TP + CIS are shown in the *right panel*. The perforated patch-clamp recording configuration was used to control the membrane potential in cells preloaded with 0.5 μM Indo 1-AM.

pine and Bay K 8644 were small in gonadotrophs, and larger in lactotrophs and somatotrophs.

To determine the $I_{K(Ca)}$ subtype activated by voltage-gated Ca^{2+} entry in somatotrophs, lactotrophs, and gonadotrophs, we used selective blockers and stimulators of BK and SK channels. The specific BK channel blockers, charybdotoxin (CTX; 100 nM), iberiotoxin (IBTX; 100 nM), and paxilline (1 μM), markedly reduced the I_K in all three cell types (Fig. 9, A–C and Table 3). In addition, the BK channel activator, NS 1619 (30 μM), increased I_K amplitude in somatotrophs

Table 2. Peak I_K in Somatotrophs, Lactotrophs, and Gonadotrophs Evoked by a 500-msec Voltage Step from −90 mV to +80 mV in Ca^{2+}-Containing and Ca^{2+}-Deficient Medium

	Ca^{2+}-Containing (pA/pF)	Ca^{2+}-Deficient (pA/pF)
Somatotrophs (19)[a]	306 ± 14	309 ± 18
Lactotrophs (16)	233 ± 16	229 ± 26
Gonadotrophs (8)	434 ± 24	442 ± 29

All currents are expressed as mean ± SEM.
[a] Numbers in parentheses indicate number of trials in each treatment group.

and lactotrophs, but not in gonadotrophs (Fig. 9D and Table 3). Unlike the BK channel blockers, the SK channel blocker, apamin, had no effect on the I_K evoked by the 100-msec Ca^{2+}-influx step in the majority of somatotrophs (n = 27) and lactotrophs (n = 11) examined. In the remaining somatotrophs and lactotrophs, apamin significantly reduced the I_K (Fig. 9E and Table 3). In all gonadotrophs examined (n = 10), apamin had no effect on the I_K evoked by the 100-msec Ca^{2+}-influx step (Fig. 9E and Table 3). This was not due to the absence of SK channels in these cells, as GnRH simulated I_{SK} in all gonadotrophs (data not shown).

In the presence of apamin and CTX, IBTX, or paxilline, no further reduction in I_K was observed when the cells were perifused with Ca^{2+}-deficient medium (data not shown). These results indicate that $I_{K(Ca)}$ activation by voltage-gated Ca^{2+} influx in somatotrophs, lactotrophs, and gonadotrophs is predominantly mediated by BK channels. It should be noted, however, that the inability of apamin to significantly reduce the I_K in a majority of the cells examined is most likely due to the relatively small current generated by its activation and not the lack of expression in each cell type. In addition, other Ca^{2+}-sensitive currents that are expressed in pituitary cells, such as Ca^{2+}-activated Cl^- channels (8, 9), may be masked by the much larger I_K in these cells.

To determine the duration of voltage-gated Ca^{2+} influx required to activate I_{BK} in each cell type, we varied the duration of the Ca^{2+}-influx step and measured the peak I_K amplitude during the subsequent 500-msec voltage step to +80 mV. To isolate BK channels from SK channels, 100 nM apamin was added to the extracellular medium. In somatotrophs, 5-msec Ca^{2+}-influx steps were sufficient to activate I_{BK}, whereas 10-msec steps were required in lactotrophs. In both cell types, the peak I_K increased progressively in response to incremental increases in the duration of the Ca^{2+}-influx step (Fig. 10, A–C). This increase was not observed in Ca^{2+}-deficient medium (Fig. 10, B and C) or in cells preloaded with BAPTA-AM (Fig. 10C). In gonadotrophs, Ca^{2+}-influx steps greater than 75 msec were required to activate I_{BK} (Fig. 10, A–C). In addition, no increase in I_{BK} activation was observed in response to further increases in the duration of voltage-gated Ca^{2+} influx (Fig. 10C). These results suggest that brief periods of voltage-gated

Table 3. Effects of Ca^{2+}-Deficient Medium and Ca^{2+}-Sensitive K^+ Channel Blockers and Activators on the Peak I_K in Somatotrophs, Lactotrophs, and Gonadotrophs

	Somatotrophs		Lactotrophs		Gonadotrophs	
	Control	Treatment	Control	Treatment	Control	Treatment
Ca^{2+}-deficient	100 ± 13	31 ± 13[a(13)]	100 ± 13	47 ± 8[a(15)]	100 ± 9	82 ± 8[a(9)]
IBTX (100 nM)	100 ± 3	36 ± 3[a(6)]	100 ± 13	36 ± 3[a(7)]	100 ± 15	78 ± 11[a(6)]
CTX (100 nM)	100 ± 22	50 ± 8[a(3)]	100 ± 23	47 ± 14[a(4)]	100 ± 5	87 ± 5[a(6)]
Paxilline (1 μM)	100 ± 18	33 ± 3[a(12)]	100 ± 10	47 ± 5[a(4)]	100 ± 4	92 ± 5[a(5)]
NS 1619 (30 μM)	100 ± 23	157 ± 26[a(4)]	100 ± 20	136 ± 23[a(5)]	100 ± 8	93 ± 9[(5)]
Apamin (100 nM)	100 ± 10	85 ± 9[a(10)]	100 ± 9	95 ± 8[a(3)]	100 ± 8	102 ± 9[(10)]

All I_K values were evoked by a two-step protocol, consisting of a 100-msec Ca^{2+}-influx step followed by a 500-test pulse to +80 mV, during which the peak I_K was monitored. All currents are normalized to the peak I_K in the absence of drug application and are expressed as mean ± SEM. Superior numbers in parentheses indicate number of trials in each treatment group.
[a] Significant difference ($P < 0.05$) compared to controls.

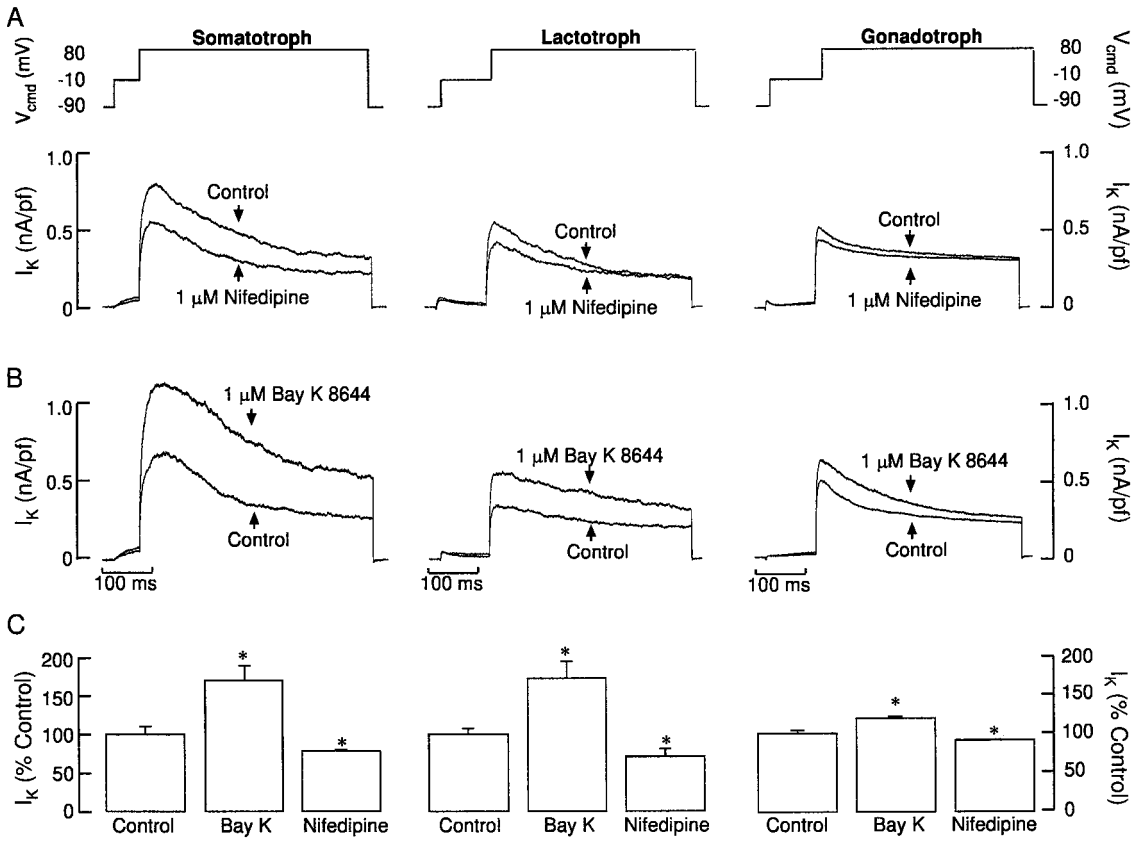

Fig. 8. Dihydropyridine Sensitivity of I_{Ca} and $I_{K\text{-}Ca}$ in Pituitary Cells

Under isolated I_K recording conditions, the effects of 1 μM nifedipine (A) and 1 μM (–)-Bay K 8644 (B) on the I_K amplitude evoked during the TP by 50-msec (somatotrophs) or 100-msec (lactotrophs and gonadotrophs) CIS were examined, and the representative current recordings are shown. C, The peak I_K amplitude during the test pulse in the presence of 1 μM nifedipine or 1 μM (−)-Bay K 8644 were normalized to the levels reached in the presence of the vehicle alone (control) and expressed as the mean ± SEM for somatotrophs (n = 3), lactotrophs (n = 5), and gonadotrophs (n = 3). *Asterisks* indicate significant differences ($P < 0.05$) compared with control values.

Ca^{2+} influx are sufficient to activate BK channels in somatotrophs and lactotrophs, whereas prolonged Ca^{2+} influx is required in gonadotrophs. Moreover, it appears that increasing the duration of voltage-gated Ca^{2+} influx recruits more BK channels in somatotrophs and lactotrophs, but not gonadotrophs.

DISCUSSION

Despite the similar origin of somatotrophs, lactotrophs, and gonadotrophs, these cells differ with respect to their pattern of basal hormone secretion. Specifically, basal gonadotropin is low compared with

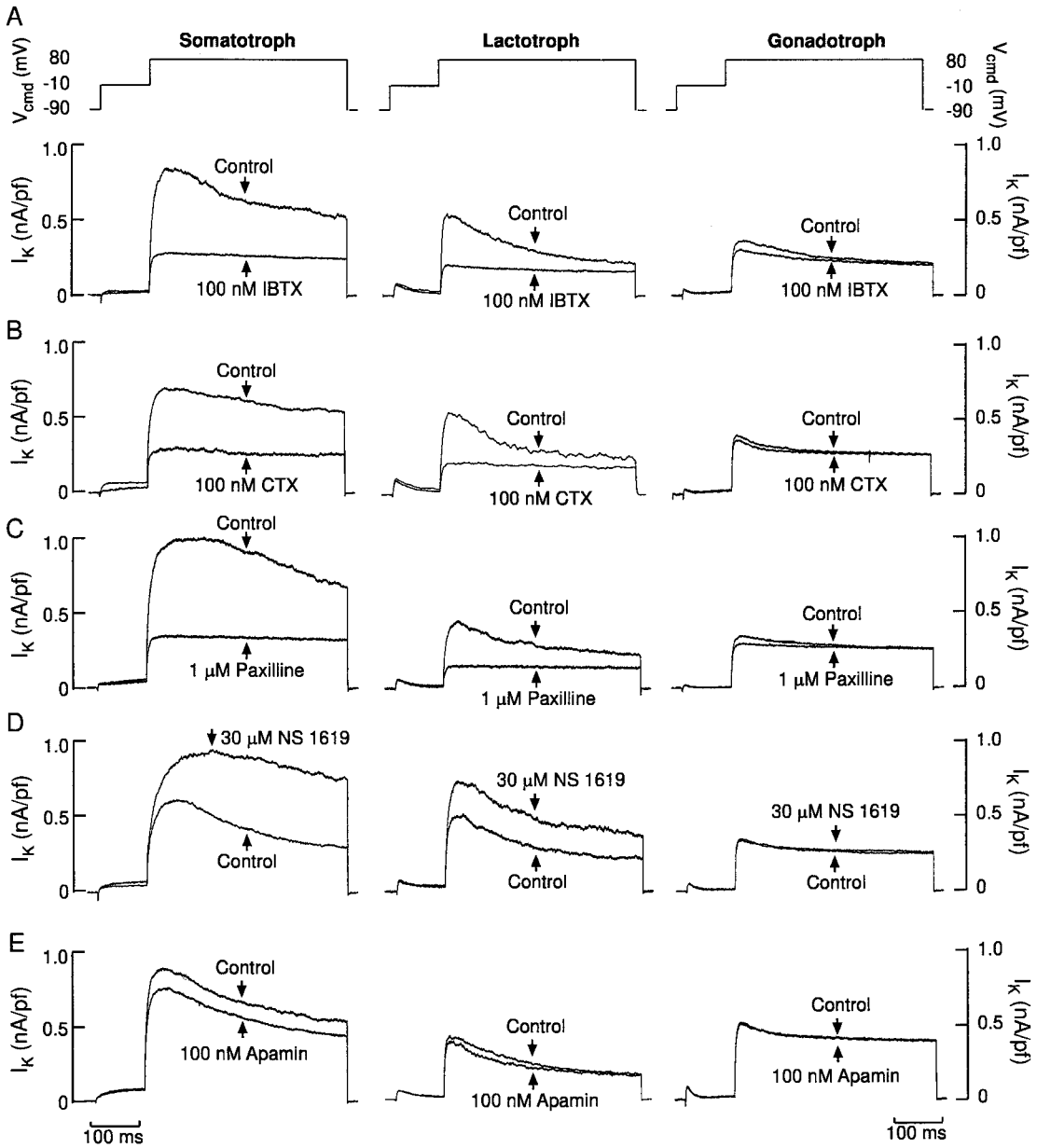

Fig. 9. Pharmacological Identification of BK Channels in Pituitary Cells

A–D, Representative current traces showing the effects of 100 nM IBTX (A), 100 nM CTX (B), 1 μM paxilline (C), 30 μM NS 1619 (D), and 100 mM apamin (E), on the I_K in somatotrophs, lactotrophs, and gonadotrophs. Averaged data for each cell type are shown in Table 3.

basal GH and PRL secretion. Earlier studies have indicated that these differences in basal hormone secretion are due to differences in spontaneous Ca^{2+} signaling among the three cell types. Unlike basal GH and PRL secretion, basal gonadotropin secretion is not dependent on the presence of extracellular Ca^{2+} or modified by increases in the extracellular Ca^{2+} concentration (4). In addition, application of voltage-gated Ca^{2+} channel blockers reduces basal GH and PRL secretion, but not basal LH or FSH secretion (4, 25). Consistent with these differences in the apparent role of voltage-gated Ca^{2+} channels in controlling basal hormone secretion, the profile of spontaneous Ca^{2+} signaling among the three cell types is also different. In rat lactotrophs and somatotrophs, spontaneous and high amplitude Ca^{2+} signals have been observed, whereas only low amplitude Ca^{2+} signals have been observed in spontaneously active rat gonadotrophs (2–4, 25, 35). These differences in the extracellular Ca^{2+} dependency and voltage-gated Ca^{2+} channel involvement in determining the pattern of basal hormone secretion and the cell type-specific patterns of spontaneous Ca^{2+} signaling suggest that there are differences in the level and/or composition of ionic

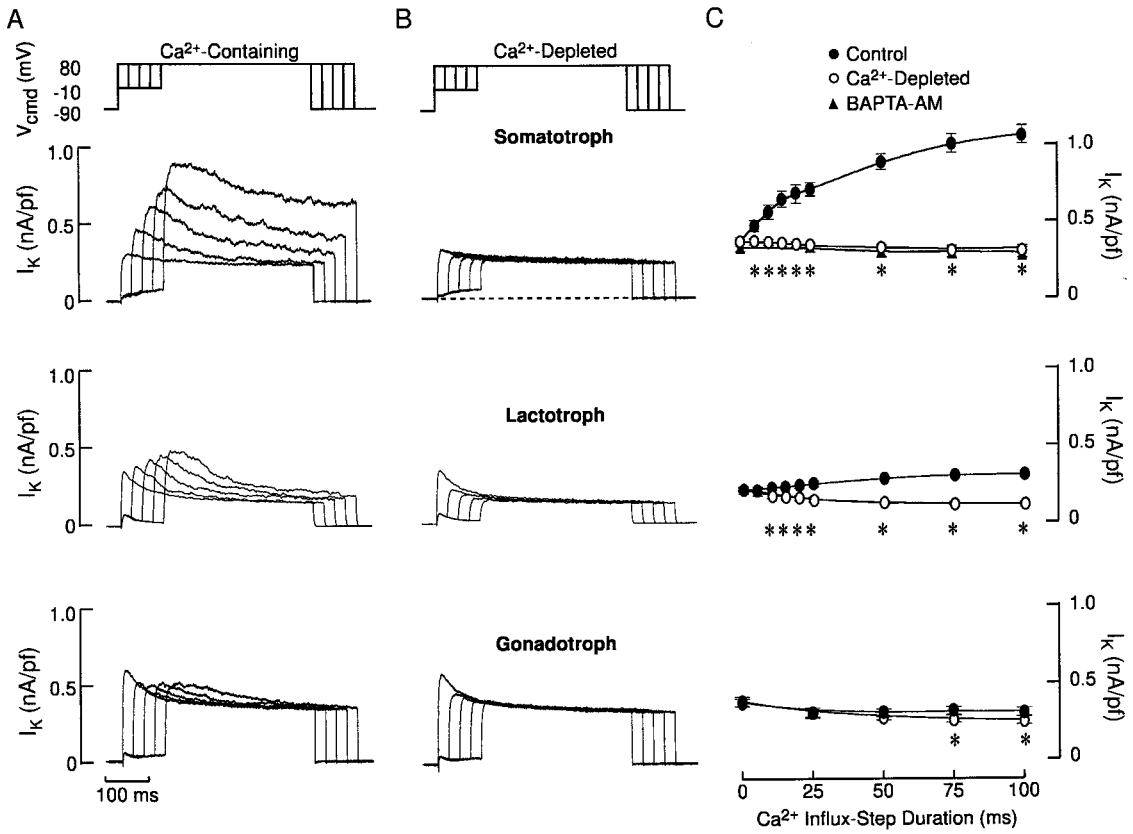

Fig. 10. Dependence of I_{BK} Activation on Duration of Voltage-Gated Ca^{2+} Influx

The effects of increasing the duration of VGCC entry on I_K activation were examined by incremental increases in the duration of Ca^{2+} influx step from 0 to 100 msec, followed by a test pulse to +80 mV for 500 msec. Representative current tracings recorded in Ca^{2+}-containing (A) or Ca^{2+}-deficient medium (B) are shown for somatotrophs (n = 13), lactotrophs (n = 12), and gonadotrophs (n = 12). C, Peak I_K amplitude (mean ± SEM) during the test pulse after 0- to 150-msec conditioning pulses in Ca^{2+}-containing medium (*filled circles*), Ca^{2+}-deficient medium (*open circles*), and in cells preloaded with BAPTA-AM (*filled triangles*). *Asterisks* indicate a significant difference compared with the peak I_K evoked in the absence of a depolarizing pulse. All experiments were performed in the presence of 100 nM apamin to block SK channel activation.

channel expression and the underlying electrical activity between rat gonadotrophs, lactotrophs, and somatotrophs.

In this study, we compared the expression levels of different ionic channels between pituitary somatotrophs, lactotrophs, and gonadotrophs. As a measure of the functional expression levels of the ionic channels in each cell type, the isolated currents were analyzed. The results indicate a quantitative rather than qualitative difference in the expression pattern for the major ionic currents in pituitary cells. All three cell types express transient and sustained voltage-gated Ca^{2+} channels, TTX-sensitive Na^+ channels, transient and delayed rectifying K^+ channels, and multiple Ca^{2+}-sensitive K^+ channel subtypes. Lactotrophs and somatotrophs exhibited low expression levels of TTX-sensitive Na^+ channels and high expression levels of BK channel compared with those observed in gonadotrophs. The expression of the transient voltage-gated K^+ channel was much higher in lactotrophs and gonadotrophs than in somatotrophs. Finally, the expression of the transient VGCCs was higher in somatotrophs than in lactotrophs and gonadotrophs.

TTX-sensitive Na^+ channels have been previously identified in gonadotrophs (10, 11), lactotrophs (12), and somatotrophs (13), as well as other pituitary cell types (14–16), indicating that these channels are common among mammalian pituitary cells. Our results extended these findings by demonstrating that the level of Na^+ channel expression is much greater in rat gonadotrophs than the other two cell types. However, it is unlikely that the difference in the expression of these channels is relevant for the control of spontaneous electrical activity (12, 17), Ca^{2+} signaling (17), and basal hormone secretion (16, 18) in these cells. The lack of TTX-sensitive Na^+ channel involvement in controlling membrane excitability and secretion is most likely due to the inactivation of a large proportion of these channels at the resting membrane potential in these cells (Fig. 1 and Ref. 10). Consistent with this, GnRH-induced transient membrane hyperpolarization in rat gonadotrophs is required to remove the steady-

state inactivation of TTX-sensitive Na^+ channels before they can contribute to action potential (AP) firing (10). This TTX sensitivity during agonist-induced AP firing in gonadotrophs is important for sustained Ca^{2+} signaling through VGCCs and the refilling of endoplasmic reticulum Ca^{2+} stores. There are, however, two exceptions. First, in a fraction of ovine gonadotrophs, these channels are responsible for AP generation (19). Second, two lactotroph subpopulations have been identified that differ with respect to their level of Na^+ channel expression, and only in lactotrophs expressing high levels of Na^+ channels did TTX application abolish basal hormone secretion (20). In the present study, we did not observe a significant difference in the level of Na^+ channel expression in the lactotrophs identified by our cell separation and identification protocol.

The expression of both inactivating and noninactivating VGCCs in rat gonadotrophs (10, 21, 22), somatotrophs (13), and lactotrophs (13), as well as immortalized pituitary cells (14, 15, 23, 24), has been well documented. The inactivating I_{Ca} is mediated by the low voltage-activated or transient (T)-type Ca^{2+} channel. Although we observed T-type I_{Ca} in all three cell types examined, it was more prominent in somatotrophs than in lactotrophs and gonadotrophs. A similar conclusion was reached in a study that directly compared VGCC expression between somatotrophs and lactotrophs. The prominent expression of T-type Ca^{2+} channels in somatotrophs is reflected by their contribution to the generation of the high amplitude $[Ca^{2+}]_i$ transients in spontaneously active somatotrophs (25), whereas its role in other native anterior pituitary cells is not known. The noninactivating I_{Ca} in pituitary cells is mediated by dihydropyridine-sensitive (L-type) and -insensitive, high-voltage activated Ca^{2+} channels (10, 21, 24, 26). Unlike the T-type Ca^{2+} channel, the current voltage-relationship of the sustained I_{Ca} in all three cell types was similar. However, the sustained I_{Ca} density was higher in somatotrophs and gonadotrophs than in lactotrophs. Despite the different densities of the sustained I_{Ca}, it is essential to the generation of both spontaneous and agonist-induced AP-driven Ca^{2+} entry in all native and immortalized pituitary cells (4, 27, 28). In addition, the L-type VGCC has been demonstrated to contribute to the regulation of basal and agonist-stimulated GH and PRL secretion, as well as agonist-induced gonadotropin secretion (28, 29).

In general, the VGCC-dependent rise in $[Ca^{2+}]_i$ is sufficient to trigger activation of several Ca^{2+}-sensitive channels. One such channel is the BK-type K^+ channel, the expression of which has been previously demonstrated in immortalized anterior pituitary cells (30–32), and native intermediate pituitary cells (33). Our studies indicate that BK channels are also expressed in native anterior pituitary cells, and that they are coupled to voltage-gated Ca^{2+}-influx in all three cell types examined. Moreover, by comparing BK channel activation between somatotrophs, lactotrophs, and gonadotrophs under identical culture and recording conditions, it was demonstrated that BK channel activation was much greater in somatotrophs than in gonadotrophs. Due to the similarities in the voltage-gated I_{Ca} density and change in $[Ca^{2+}]_i$ evoked by the Ca^{2+}-influx step between the three cell types, the differences in I_{BK} activation are most likely due to differences in BK channel expression levels.

In other excitable cells, the colocalization of BK channels with VGCCs facilitates spike repolarization, which limits AP-driven Ca^{2+} influx. BK channel activation can also influence the frequency of AP-driven $[Ca^{2+}]_i$ transients by slowing the pacemaker depolarization (34). In native anterior pituitary cells, the role of BK channels in shaping the frequency and duration of AP-driven Ca^{2+} entry is not known. Based on our results, we would expect that the relatively high levels of BK channel expression in somatotrophs and lactotrophs would limit AP-driven Ca^{2+} influx compared with that in gonadotrophs, which exhibit the lowest levels of BK channel expression. However, previous studies have demonstrated that the duration of the AP waveform is longer in somatotrophs and lactotrophs (100–500 msec) than in gonadotrophs (10–100 msec) (10, 35–37). In addition, both the amplitude and duration of the spontaneous, extracellular Ca^{2+}-dependent $[Ca^{2+}]_i$ transients are greater in somatotrophs and lactotrophs than in gonadotrophs (2). It is unlikely that the prolonged duration of AP-driven Ca^{2+} entry in somatotrophs and lactotrophs is due to the inability of AP firing to activate BK channels, as short Ca^{2+} influx steps ($<$25 msec) were sufficient to activate I_{BK} in both cell types (Fig. 10). Thus, whether BK channels have an atypical role in regulating the pattern of AP firing and Ca^{2+} signaling in anterior pituitary cells requires further studies.

All three pituitary cell types also express SK channels (31, 38–40). However, in gonadotrophs SK channels did not appear to be coupled to voltage-gated Ca^{2+} influx under the conditions used in our study. Similarly, voltage-gated Ca^{2+} influx activated SK channels in only a small fraction of the somatotrophs and lactotrophs examined. This may be due to the lack of SK channel expression in some somatotroph and lactotroph subpopulations. Consistent with this, activation of Ca^{2+}-mobilizing TRH receptors leads to activation of SK channels and the concomitant membrane hyperpolarization in GH_3 cells (32), but only a small fraction of lactotrophs exhibited a similar response (41). It is also possible that the SK channels in pituitary cells, as in other cell types (reviewed in Ref. 42), are colocalized with intracellular Ca^{2+} release sites and can be activated only by Ca^{2+}-mobilizing receptors, sustained voltage-gated Ca^{2+} entry, and/or Ca^{2+}-induced Ca^{2+} release. For example, in GnRH-secreting neurons, agonist-induced Ca^{2+} mobilization and the concomitant increase in firing frequency are needed to activate SK channels (43). Similarly, in GH_3 cells, SK channel activation requires high-frequency firing, prolongation of APs by voltage-dependent K^+

channel inhibitors, or release of Ca^{2+} from intracellular Ca^{2+} stores (31). The lack of a detectable apamin-sensitive I_K in a majority of the three cell types examined may also be due to its relatively small size compared with the voltage-gated I_K and the I_{BK} in these cells. Other Ca^{2+}-sensitive channels, such as Cl^- channels [known to be expressed in AtT-20 immortalized cells (8) and native lactotrophs (9)] may also be masked.

Several different voltage-gated K^+ channel subtypes have been identified and characterized in somatotrophs, lactotrophs, and gonadotrophs (44, 45), as well as immortalized pituitary cells (14, 15). One such channel is the transient, 4-AP-sensitive (A-type) K^+ channel. Direct comparison of the three anterior pituitary cells examined in this study indicates that the expression level of the A-type K^+ channel is much higher in lactotrophs and gonadotrophs than in somatotrophs. In contrast, these channels have been observed in ovine somatotrophs and may contribute to AP firing and hormone secretion (46). The participation of the A-type K^+ channel in regulating AP firing in other anterior pituitary cell types is also unclear. In rat lactotrophs, for example, they do not appear to participate in AP generation (37), which may be due to their prominent inactivation at the resting membrane potential in these cells. The participation of the delayed rectifying K^+ channel during AP firing in anterior pituitary cells is also not clear. In immortalized cells, inhibition of this channel by tetraethylammonium (TEA) increased the duration of the AP (37) and the amplitude of the spontaneous $[Ca^{2+}]_i$ transients (47), whereas in native rat lactotrophs, TEA did not alter the pattern of AP firing (37). Further studies are required to elucidate the role of both the A-type and delayed rectifying K^+ channels in the regulation of AP-driven Ca^{2+} signaling in the different anterior pituitary cell types.

Our study was focused on the ionic channels involved in the formation of the AP waveform. Other channels have also been identified in pituitary cells, and they contribute to pacemaking activity in these cells. For example, a TTX-insensitive I_{Na} has been observed in somatotrophs (48) and lactotrophs (12) and is critical for maintaining the membrane potential near the threshold for AP firing. In addition, inward rectifier K^+ channels, including the erg-like K^+ channel, have been identified in both native (36, 49) and immortalized pituitary cells (50). They contribute to the regulation of both spontaneous and agonist-modulated AP-driven Ca^{2+} entry. The M-type K^+ channel has also been identified in pituitary cells (41), as well as several ATP-gated P2X receptor channels (51). Preliminary data also suggested the presence of cyclic nucleotide-gated channels in pituitary cells, which may contribute to pacemaking activity (25).

In summary, we examined the cell-type specific expression of the major ionic currents in somatotrophs, lactotrophs, and gonadotrophs that contribute to AP-driven Ca^{2+} entry. All experiments were done under identical experimental conditions on cells from randomly cycling adult female rats. This allowed us to directly compare the expression levels and voltage-dependent properties of several ionic channels between the three anterior pituitary cell types. Our results demonstrate that, although these cells originate from the same precursor cell, they exhibit cell type-specific patterns of ionic channel expression. In particular, the marked differences in BK channel and transient (A-type) K^+ channel expression between the three cell types are excellent candidates for future investigations into the cell type-specific patterns of spontaneous and receptor-controlled membrane excitability, Ca^{2+} signaling, and hormone secretion.

MATERIALS AND METHODS

Pituitary Cell Culture and Cell Identification

Anterior pituitary glands were excised from female Sprague Dawley rats (Taconic Farms, Inc., Germantown, NY) and dispersed into single cells using a trypsin/DNase (Sigma, St. Louis, MO) cell dispersion procedure as described previously (51). Enriched somatotroph (>90%, estimated by immunocytochemistry using specific antibodies provided by the National Hormone and Pituitary Program and Dr. Parlow) and lactotroph (>65%) populations were obtained using a discontinuous Percoll density-gradient cell-separation procedure as described previously (51). Pituitary somatotrophs and lactotrophs were further identified using a combination of cell separation techniques, their distinct morphology, and responses to GHRH and TRH, respectively. Gonadotrophs were initially identified by their distinct morphology and subsequent to experimentation by their unique oscillatory I_K and/or $[Ca^{2+}]_i$ response to GnRH, the specific agonist for these cells.

Electrophysiological Recordings

Voltage-gated I_{Na} and I_{Ca} were measured using regular whole-cell recording techniques, whereas voltage-gated I_K and $I_{K(Ca)}$ were measured using perforated-patch recording techniques (52). All voltage-clamp recordings were performed at room temperature using an Axopatch 200 B patch-clamp amplifier (Axon Instruments, Foster City, CA) and were low-pass filtered at 2 kHz. A series resistance of < 20 MΩ was reached 10 min after the formation of a gigaohm seal (seal resistance > 5 GΩ). When necessary, series resistance compensation was optimized and all current recordings were corrected for linear leakage and capacitance using a P/−N procedure. An average membrane capacitance (C_m) of 4.6 ± 0.2 pF (n = 48), 5.9 ± 0.2 pF (n = 47), and 7.5 ± 0.2 pF (n = 68) was recorded in somatotrophs, lactotrophs, and gonadotrophs, respectively. Pulse generation and data acquisition were done with a PC equipped with a Digidata 1200 A/D interface in conjunction with Clampex 8 (Axon Instruments).

Simultaneous Measurement of $[Ca^{2+}]_i$ and I_K

To simultaneously monitor $[Ca^{2+}]_i$ and I_K the cells were incubated for 15 min at 37 C in phenol red-free medium 199 containing Hanks' salts, 20 mM sodium bicarbonate, 20 mM HEPES, and 0.5 μM indo-1 AM (Molecular Probes, Inc., Eugene, OR). The coverslips with cells were then washed twice with modified Krebs-Ringer's solution containing (in mM): 120 NaCl, 4.7 KCl, 2.0 $CaCl_2$, 2 $MgCl_2$, 0.7 $MgSO_4$, 10

HEPES, 10 glucose (pH adjusted to 7.4 with NaOH) and mounted on the stage of an inverted epifluorescence microscope (Nikon, Melville, NY). A photon counter system (Nikon) was used to simultaneously measure the intensity of light emitted at 405 nm and at 480 nm after excitation at 340 nm. Background intensity at each emission wavelength was corrected. Perforated-patch recording techniques (see above) were used to control the membrane potential and monitor I_K in the voltage-clamp recording mode. The data were digitized at 4 kHz using a PC equipped with the Clampex 8 software package in conjunction with a Digidata 1200 A/D converter (Axon Instruments). The $[Ca^{2+}]_i$ was calibrated *in vivo* as described by Kao (53). Briefly, R_{min} was determined by exposing the cells to 10 μM Br-A23187 in the presence of Krebs-Ringer's solution with 2 mM EGTA and 0 mM Ca^{2+} for 60 min; 15 mM Ca^{2+} was then added to determine R_{max}. The values used for R_{min}, R_{max}, $S_{f,480}/S_{b,480}$, and dissociation constant (K_d) were 0.677, 2.9, 2.473, and 230 nM, respectively.

Chemicals and Solutions

For all I_K recordings, the extracellular medium contained modified Krebs-Ringer salts containing (in mM): 120 NaCl, 4.7 KCl, 2.0 $CaCl_2$, 2 $MgCl_2$, 0.7 $MgSO_4$, 10 HEPES, 10 glucose and 1 μM TTX (pH adjusted to 7.4 with NaOH) and the pipette solution contained (in mM): 50 KCl, 90 K-aspartate, 1 $MgCl_2$, and 10 HEPES (pH adjusted to 7.2 with KOH). To isolate the voltage-gated I_K, the extracellular medium contained 200 μM Cd^{2+} and 100 μM TTX. In some experiments, extracellular Ca^{2+} was replaced with equimolar Mg^{2+}. To isolate I_{Na} and I_{Ca}, conventional whole-cell recording techniques were used as previously described (52). The extracellular medium contained Krebs-Ringer's solution with 20 mM TEA and 2 mM $CaCl_2$ (pH adjusted to 7.4 with NaOH) and the pipette contained (in mM); 120 CsCl, 20 TEA-Cl, 4 $MgCl_2$, 10 EGTA, 9 glucose, 20 HEPES, 0.3 Tris-GTP, 4 Mg-ATP, 14 $CrPO_4$, and 50 U/ml creatine phosphokinase (pH adjusted to 7.2 with Tris base). To isolate the I_{Na} or I_{Ca}, 200 μM Cd^{2+} or 1 μM TTX was added to extracellular medium, respectively. All reported membrane potentials were corrected on line for a liquid junction potential of 10 mV between the pipette and bath solution, except for I_{Na} and I_{Ca}, which required a correction of 7 mV. The bath contained $<$500 μl of saline and was continuously perfused at a rate of 2 ml/min using a gravity-driven superfusion system. Stock solutions of TTX, isobutylmethylxanthine, and CTX were prepared in double-distilled, deionized water, whereas stock solutions of paxilline, nifedipine and S(–)-Bay K 8644 were prepared in dimethylsulfoxide. All chemicals were obtained from Sigma-Aldrich Corp. (St. Louis, MO). In some experiments, the cells were loaded with 1 μM BAPTA-AM (Sigma-Aldrich Corp.) at 37 C for 45 min.

Data Analysis

Data analysis was performed using Clampfit (Axon Instruments). In some cases, the current-voltage relations were fit with a single Boltzmann relation: $I/I_{max} = I_{max} + \exp[(E - E_{1/2})/k]$; where I_{max} is the maximum current, E is the command potential, $E_{1/2}$ is the membrane potential at which there is 50% of the maximal current, and k is the slope factor. The results shown are typical tracing or means ± SEM for at least five recordings. Differences between groups were considered to be significant with $P < 0.05$ or higher, calculated by paired t test or ANOVA, followed by Fisher's least significant differences test.

Acknowledgments

Received July 10, 2000. Re-revision received March 27, 2001. Accepted April 2, 2001.

Address requests for reprints to: Dr. Fredrick Van Goor, c/o Stanko S. Stojilkovic, Section on Cellular Signaling, Endocrinology and Reproduction Research Branch, National Institute of Child Health and Human Development, National Institutes of Health, Building 49, Room 6A36, 49 Convent Drive, Bethesda, Maryland 20892-4510. E-mail: stankos@helix.nih.gov.

REFERENCES

1. Burrows HL, Douglas KR, Seasholtz AF, Camper SA 1999 Genealogy of the anterior pituitary gland: tracing a family tree. Trends Endocrinol Metabol 10:343–352
2. Stojilkovic SS, Catt KJ 1992 Calcium oscillations in anterior pituitary cells. Endocr Rev 13:256–280
3. Kwiecien R, Hammond C 1998 Differential management of Ca^{2+} oscillations by anterior pituitary cells: a comparative overview. Neuroendocrinology 68:135–151
4. Stojilkovic SS, Izumi S-I, Catt KJ 1988 Participation of voltage-sensitive calcium channels in pituitary hormone secretion. J Biol Chem 263:13054–13061
5. Bluet-Pajot M-T, Epelbaum J, Gourdji D, Hammond C, Kordon C 1998 Hypothalamic and hypophyseal regulation of growth hormone secretion. Cell Mol Neurobiol 18:101–123
6. Freeman ME, Kanicska B, Lernat A, Nagy G 2000 Prolactin: structure, function, and regulation of secretion. Physiol Rev 80:1523–1631
7. Stojilkovic SS, Reinhart J, Catt KJ 1994 GnRH receptors: structure and signal transduction pathways. Endocr Rev 15:462–499
8. Korn SJ, Bolden A, Horn R 1991 Control of action potentials and Ca^{2+} influx by the Ca^{2+}-dependent chloride current in mouse pituitary cells. J Physiol 439:423–437
9. Sator P, Dufy-Barbe L, Vacher P, Dufy B 1992 Calcium-activated chloride conductance of lactotrophs; comparison of activation in normal and tumoral cells during thyrotropin-releasing-hormone stimulation. J Membr Biol 126:39–49
10. Tse A, Hille B 1993 Role of voltage-gated Na^+ and Ca^{2+} channels in gonadotropin-releasing hormone-induced membrane potential changes in identified rat gonadotropes. Endocrinology 132:1475–1481
11. Marchetti C, Childs GV, Brown AM 1987 Membrane currents of identified isolated rat corticotropes and gonadotropes. Am J Physiol 252:E340–E346
12. Sankaranarayanan S, Simasko SM 1996 A role for a background sodium current in spontaneous action potentials and secretion from rat lactotrophs. Am J Physiol 271:C1927–C1934
13. Lewis DL, Goodman MB, St.John PA, Barker JL 1988 Calcium currents and fura-2 signals in fluorescence-activated cell sorted lactotrophs and somatotrophs of rat anterior pituitary. Endocrinology 123:611–621
14. Dubinsky JM, Oxford GS 1984 Ionic currents in two strains of rat anterior pituitary tumor cells. J Gen Physiol 83:309–339
15. Bosma MM, Hille B 1992 Electrophysiological properties of a cell line of the gonadotrope lineage. Endocrinology 130:3411–3420
16. Mason WT, Skidar SK 1988 Characterization of voltage-gated sodium channels in ovine gonadotrophs: relationship to hormone secretion. J Physiol 399:493–551
17. Stojilkovic SS, Kukuljan M, Iida T, Rojas E, Catt KJ 1992 Integration of cytoplasmic calcium and membrane potential oscillations maintains calcium signaling in pituitary gonadotrophs. Proc Natl Acad Sci USA 89:4081–4085
18. Mason WT, Rawlings SR 1988 Whole-cell recordings of ionic currents in bovine somatotrophs and their involvement in growth hormone secretion. J Physiol 405: 577–593

19. Heyward PM, Chen C, Clarke IJ 1995 Inward membrane currents and electrophysiological responses to GnRH in ovine gonadotropes. Neuroendocrinology 61:609–621
20. Horta J, Hiriart M, Gota G 1991 Differential expression of Na channels in functional subpopulations of rat lactotropes. Am J Physiol 261:C865–871
21. Stutzin A, Stojilkovic SS, Catt KJ, Rojas E 1989 Characteristics of two types of calcium channels in rat pituitary gonadotrophs. Am J Physiol 257:C865–C874
22. Marchetti C, Childs GV, Brown AM 1990 Voltage-dependent calcium currents in rat gonadotropes separated by centrifugal elutriation. Am J Physiol 258:E589–E596
23. Simasko SM, Weiland GA, Oswald RE 1988 Pharmacological characterization of two calcium currents in GH_3 cells. Am J Physiol 254:E328–E336
24. Kwiecien R, Robert C, Cannon R, Vigues S, Arnoux A, Kordon C, Hammond C 1998 Endogenous pacemaker activity of rat tumour somatotrophs. J Physiol 508: 883–905
25. Tomic M, Koshimizu T, Yuan D, Andric SA, Zivadinovic D, Stojilkovic SS 1999 Characterization of a plasma membrane calcium oscillator in rat pituitary somatotrophs. J Biol Chem 274:35693–35702
26. Kuryshev YA, Childs GV, Ritchie AK 1995 Three high threshold calcium channel subtypes in rat corticotropes. Endocrinology 136:3916–3924
27. Mollard P, Theler J-M, Guerineau N, Vacher P, Chiavaroli C, Schlegel W 1994 Cytosolic Ca^{2+} of excitable pituitary cells at resting potentials is controlled by steady state Ca^{2+} currents sensitive to dihydropyridines. J Biol Chem 269:25158–25164
28. Stojilkovic SS, Iida T, Virmani MA, Izumi S-I, Rojas E, Catt KJ 1990 Dependence of hormone secretion on activation-inactivation kinetics of voltage-sensitive Ca^{2+} channels in pituitary gonadotrophs. Proc Natl Acad Sci USA 87:8855–8859
29. Cota G, Hiriart M, Horta J, Torres-Escalante JL 1990 Calcium channels and basal prolactin secretion in single male rat lactotropes. Am J Physiol 259:C949–959
30. Shipston MJ, Kelly JS, Antoni FA 1996 Glucocorticoids block protein kinase A inhibition of calcium-activated potassium channels. J Biol Chem 271:9197–9200
31. Lang DG, Ritchie AK 1990 Tetraethylammonium blockade of apamin-sensitive and insensitive Ca^{2+}-activated K^+ channels in a pituitary cell line. J Physiol 425:117–132
32. Ritchie AK 1987 Thyrotropin-releasing hormone stimulates a calcium-activated potassium current in a rat anterior pituitary cell line. J Physiol 385:611–625
33. Kehl SJ, Wong K 1996 Large-conductance calcium-activated potassium channels of cultured rat melanotrophs. J Membr Biol 150:219–230
34. Sah P 1996 Ca^{2+}-activated K^+ currents in neurons: types, physiological roles and modulation. Trends Neurosci 19:150–154
35. Li Y-X, Rinzel J, Vergara L, Stojilkovic SS 1995 Spontaneous electrical and calcium oscillations in pituitary gonadotrophs. Biophys J 69:785–795
36. Sims SM, Lussier BT, Kraicer J 1991 Somatostatin activates an inwardly rectifying K^+ conductance in freshly dispersed rat somatotrophs. J Physiol 441:615–637
37. Sankaranarayanan S, Simasko SM 1998 Potassium channel blockers have minimal effect on repolarization of spontaneous action potentials in rat pituitary lactotropes. Neuroendocrinology 68:297–311
38. Kukuljan M, Stojilkovic SS, Rojas E, Catt KJ 1992 Apamin-sensitive potassium channels mediate agonist-induced oscillations of membrane potential in pituitary gonadotrophs. FEBS Lett 301:19–22
39. Tse A, Hille B 1992 GnRH-induced Ca^{2+} oscillations and rhythmic hyperpolarizations of pituitary gonadotropes. Science 255:462–464
40. Ritchie AK 1987 Two distinct calcium-activated potassium currents in a rat anterior pituitary cell line. J Physiol 385:591–609
41. Sankaranarayanan S, Simasko SM 1996 Characterization of an M-like current modulated by thyrotropin-releasing hormone in normal rat lactotrophs. J Neurosci 16:1668–1678
42. Berridge MJ 1998 Neuronal calcium signaling. Neuron 21:13–26
43. Van Goor F, Krsmanovic LZ, Catt KJ, Stojilkovic SS 1999 Coordinate regulation of gonadotropin-releasing hormone neuronal firing patterns by cytosolic calcium and store depletion. Proc Natl Acad Sci USA 96:4101–4106
44. Herrington J, Lingle CJ 1994 Multiple components of voltage-dependent potassium current in normal rat anterior pituitary cells. J Neurophysiol 72:719–729
45. Lingle CJ, Sombati S, Freeman ME 1986 Membrane currents in identified lactotrophs of rat anterior pituitary. J Neurosci 6:2995–3005
46. Chen C, Heyward P, Zhang J, Wu D, Clarke IJ 1994 Voltage-dependent potassium currents in ovine somatotrophs and their function in growth hormone secretion. Neuroendocrinology 59:1–9
47. Charles AC, Piros ET, Evans CJ, Hales TG 1999 L-type Ca^{2+} channels and K^+ channels specifically modulate the frequency and amplitude of spontaneous Ca^{2+} oscillations and have distinct roles in prolactin release in GH_3 cells. J Biol Chem 274:7508–7515
48. Takano K, Takei T, Teramoto A, Yamashita N 1996 GHRH activates a nonslective cation current in human GH-secreting adenoma cells. Am J Physiol 270: E1014–E1057
49. Schafer R, Wulfsen I, Behrens S, Weinsberg F, Bauer CK, Schwartz JR 1999 The erg-like potassium current in rat lactotrophs. J Physiol 518:401–416
50. Bauer CK 1998 The erg inwardly rectifying K^+ current and its modulation by thyrotrophin-releasing hormone in giant clonal rat anterior pituitary cells. J Physiol 510: 63–70
51. Koshimizu T, Tomic M, Wong AOL, Zivadinovic D, Stojilkovic SS 2000 Characterization of purinergic receptors and receptor-channels expressed in anterior pituitary cells. Endocrinology 141:4091–4099
52. Van Goor F, Krsmanovic LZ, Catt KJ, Stojilkovic SS 1999 Control of action potential-driven calcium influx in GT1 neurons by the activation status of sodium and calcium channels. Mol Endocrinol 13:587–603
53. Kao JPY 1994 Practical aspects of measuring $[Ca^{2+}]$ with fluorescent indicators. Methods Cell Biol 40:155–181
54. Van Goor F, Zivadinovic D, Wong AOL, Stojilkovic SS 2000 Calcium-activated, voltage-dependent K^+ (BK) channels account for differences in the spiking pattern between spontaneously active rat somatotrophs, lactotrophs, and gonadotrophs. 30th Annual Meeting of the Society for Neuroscience, New Orleans, LA (Abstract 689.9)